There were no significant differences between the groups at baseline in any patient characteristics or baseline assessments, including age, gender, minimal joint space, Harris Hip Score and self reported pain and function.

Conclusions: Cumulative survival without THR surgery were significantly higher for patients going through a both a supervised exercise program and patient education compared to patients going through patient education only, despite the fact that there were no significant differences in baseline characteristics between the two groups. The findings of this study suggest that supervised exercises in addition to patient education may postpone the need for THR surgery in patients with hip osteoarthritis.

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WALKING LOADING AT THE KNEE PREDICTS MRI-DERIVED CARTILAGE THICKNESS CHANGES IN MEDIAL COMPARTMENT KNEE OSTEOARTHRITIS

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Purpose: Knee osteoarthritis (OA) occurs in a substantial portion of the population over the age of 50. However, there is limited information on the underlying causes of OA and the reasons why the rate of disease progression varies between patients. In medial compartment knee OA, the peak knee adduction moment has been reported to predict disease progression as evaluated by radiographic JSW. However, mechanistic interpretation of radiographic measures of cartilage thinning is limited, and it is difficult to distinguish between femoral and tibial cartilage loss as well as assess if changes occur in specific walking load-bearing regions of the knee joint. Magnetic resonance (MR) imaging allows the investigation of specific regional changes in cartilage. We tested the hypothesis that baseline knee loading during gait predicts local MRI-derived cartilage loss in the medial compartment in patients with knee OA at a five year follow-up. Further, we hypothesized that increases in KAM over 5 years lead to focal increased cartilage loss.

Methods: Fourteen medial compartment OA knees (6 male, 8 female; age: 64.2±8.2 yrs) with an average baseline Kellgren-Lawrence grade of 2 were tested twice using MRI and gait analysis, with an average time between testing of 55 months following written consent in accordance with the Institutional Review Board. MR images were acquired with a 1.5T scanner (General Electric) using a 3D spoiled gradient-echo sequence in the sagittal plane. Images were manually segmented and 3D cartilage thickness maps were created. For local thickness analysis, the weight-bearing medial femoral cartilage was divided into three sub-regions: external, central, and internal. Similarly, the medial tibia cartilage was divided in five sub-regions: central, anterior, external, posterior, and internal. Subjects performed 3 walking trials at a self-selected normal speed in their personal shoes. Kinematic and kinetic data were captured using a 10-camera optoelectronic motion capture system (Qualisys), and the first peak knee adduction moment was calculated using inverse dynamics. Linear regression was used to test for a correlation between changes in cartilage thickness and joint loading. The level of significance was set at <0.05, with trends defined as <0.1.

Table 1. Correlation coefficients for associations between cartilage thinning and peak KAM

	Medial weight-bearing femur				Medial tibia					
	External	Central	Internal	Total	Central	Anterior	External	Posterior	Internal	Total
Baseline KAM										
R	-0.636	-0.514	0.158	-0.499	-0.487	-0.286	-0.189	-0.560	-0.247	-0.426
P-value	0.01	0.06	0.59	0.07	0.08	0.32	0.52	0.04	0.39	0.13
ΔKAM										
R	-0.602	-0.74	0.129	-0.630	-0.297	-0.455	-0.435	-0.254	-0.038	-0.335
P-value	0.02	0.00	0.66	0.02	0.30	0.10	0.12	0.38	0.90	0.24

Results: At the five-year follow-up, baseline peak KAM significantly predicted cartilage thinning in the medial femoral external region (P = 0.01), with trends towards correlations for the medial central (P = 0.06) and total medial (P = 0.07) weight-bearing regions (Table 1). Only one significant association between the baseline peak KAM and thinning was seen on the medial tibia posterior region (P = 0.04), with a trend for the central medial tibia (P = 0.08). When the change in adduction moment (ΔKAM) was considered there was a broader range of regions on the medial femur where cartilage thickness changes over 5 years were related to changes in KAM. An increase in ΔKAM was significantly associated with an increase in thinning for the external, central, and total weight-bearing medial femur (P = 0.02, 0.002, and 0.02, respectively). No significant associations were seen on the medial tibia.

Conclusions: These results suggest that over 5 years baseline KAM can predict OA progression, and suggest that the effect of the KAM on cartilage thinning may be region dependent, with more associations found on the medial weight-bearing femur. Further, an increased loss of cartilage thickness is associated with an increase in dynamic joint loading over 5 years, which may further accelerate the rate of OA progression, and suggest the KAM should be reduced to slow disease progression.

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GENOME WIDE EXPRESSION PROFILING OF NORMAL, RHEUMATOID AND OSTEOARTHRITIC SYNOVIAL STEM CELLS

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Purpose: Within synovial joints, damage to the articular cartilage surface is thought to be irreversible in part due to the lack of stem cells within the cartilage. Interestingly, however, the synovial lining of the joint and the synovial fluid itself contains adult stem cells similar in behaviour and potential to mesenchymal stem cells (MSCs). *In vitro* these synovial MSCs (sMSCs) display increased chondrogenic potential compared to fat and bone marrow MSCs, and are indeed present in normal joints, with greater numbers of stem cells within arthritic joints. While it does not appear that these cells are able to affect repair within individuals with osteoarthritis (OA) or rheumatoid arthritis (RA), there is growing evidence from animal models that sMSCs and/or synovial tissue can contribute to articular cartilage repair *in vivo*.

Methods: In our current study, synovial fluid MSCs were isolated and characterized from normal, OA and RA knee joints diagnosed using the American College of Rheumatology Standards and analyzed at the genome-wide expression levels using micro-array technology. Nine genes of interest were identified for further study and validated using qPCR in 5 normal, 5 OA and 5 RA individuals. Furthermore, sMSCs were analyzed from five patients with early osteoarthritis identified through arthroscopic examination and were found to show similar expression patterns to sMSCs collected from advanced OA and RA joints.

Results: A number of genes were found to be differentially regulated between normal, OA and RA sMSCs (Figure 1). Cartilage oligomeric matrix protein (COMP), Cathepsin K (CTSK), Iterleukin-1 receptor type I (IL1R1), Stromelysin-1 (MMP3), SPARC-related modular calcium-binding protein 2 (SMOC2), Osteopontin (SPP1), Substance-P receptor (TACR1), Thrombospondin-2 (THBS2) and Tenascin-X (TNXB) expression levels were all significantly different between normal and arthritic sMSCs as well as early OA sMSCs.

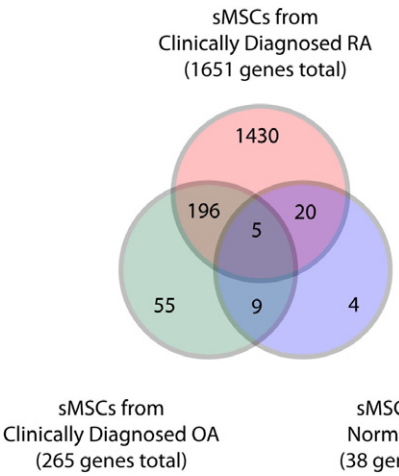

Fig. 1. Representation of differentially regulated genes in sMSC population. sMSCs from normal, OA and RA synovial fluid (n = 2 each) were subjected to microarray analysis. All genes shown are significantly expressed p > 0.05.

Conclusions: The resulting data strongly suggests that the synovial stem cell biology is altered during arthritis at the genome wide expression level, even in OA cases, where joint space narrowing is not

present. It is quite possible that these sMSCs are extremely sensitive to changes/damage to the joint environment and therefore may be exploited as a sentinel for early detection of OA.

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NORMAL AND OSTEOARTHRITIC SYNOVIAL STEM CELL-DERIVED TISSUE-ENGINEERED CONSTRUCTS RESPOND TO MECHANICAL STIMULUS FOLLOWING CHONDROGENIC DIFFERENTIATION

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Purpose: Tissue-engineering (TE) comprises cells, a scaffold, and appropriate signals. In the context of TE of articular cartilage for treatment of osteoarthritis (OA), synovial tissue-derived mesenchymal stem cells (sMSCs) have been demonstrated to be particularly suitable. These MSCs are present in the synovial fluid and their numbers increase dramatically with the onset of OA. Recently scaffold-free tissue-engineered constructs (TECs) from sMSC monolayers treated with ascorbic acid (AA) were developed in our laboratory. These TECs retained their chondrogenic potential after transplantation into chondral defects within a porcine model. However, the implanted TECs exhibited suboptimal healing as they failed to take on a completely hyaline phenotype based on the presence of fibrous tissue at the surface of the repair sites. Therefore, the objective of this study is to modify the present TEC technology through the induction of chondrogenic differentiation using biochemical and mechanical signals.
Methods: Human sMSCs were derived from donor synovial tissue and synovial fluid and isolated using magnetic purification. When the cells reached confluence they were treated with either chondrogenic media containing TGF-β3, BMP-2, AA, and dexamethasone, media supplemented with only AA (TEC), or control media, for a period of 14 days. The chondrogenic TECs (cTECs) and AA-treated TECs were then subjected to mechanical compression for 24 hours with a loading protocol of 1 MPa at 1 Hz for 1 minute and 14 minutes of no load using a Flexcell system. Non-loaded TECs were maintained in parallel as controls. After loading, the remaining TECs and cultures were harvested for analysis. Analysis included RT-qPCR, histology & immunohistochemistry, and mechanical testing.
Results: Mechanical loading differentially regulated several genes commonly used as markers of chondrogenic differentiation in both the cTECs and AA-treated TECs (Fig. 1). Following mechanical compression, mRNA expression of SOX-9 and aggrecan are upregulated, while type-II collagen expression is highly variable. As well, histological assessment indicated significant differences in composition between the cTECs and AA-treated TECs (Fig. 2). The cTECs appear to have increased collagen content and are more sparsely populated with cells. Similar observations were made for TECs formed using MSCs from OA tissue, however the OA TECs tended to be quite variable in terms of their various properties.
Conclusions: The results indicate that the modified TEC protocol is more effective than the original protocol for producing TECs that exhibit characteristics similar to that of native articular cartilage, such as increased collagen content and relatively low cell density. Optimization of the protocol (e.g. loading regimen, timing of biochemical and mechanical cues, etc.) could lead to further improved TEC properties. However, additional modifications are necessary to achieve similar results with OA TECs.

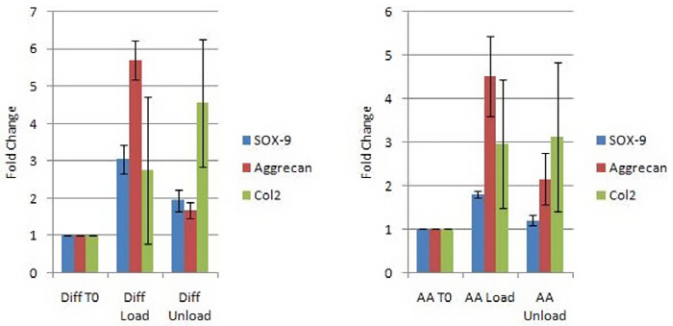

Fig. 1. Relative mRNA expression of chondrogenic markers in loaded and unloaded cultures treated with either chondrogenic media (left) or media containing AA (right), compared to initial controls.

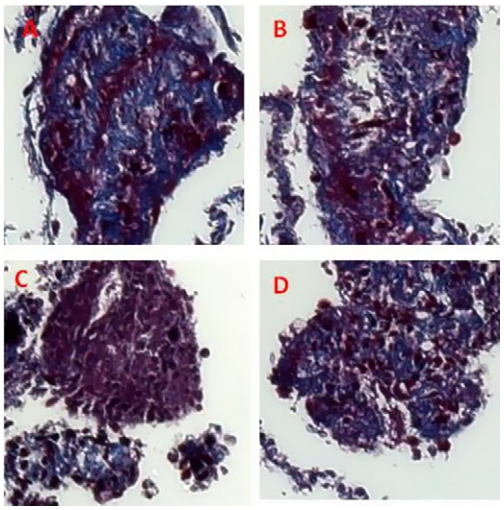

Fig. 2. Sections of cTECs (A,B) and AA-treated TECs (C,D) stained with Masson's trichrome (blue: collagen; red/purple: cytoplasm; black: nuclei). TECs were either loaded (A,C) or unloaded (B,D).

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A QUANTITATIVE PROTEOMICS APPROACH FOR STUDYING THE CHONDROGENIC DIFFERENTIATION PROCESS OF MESENCHYMAL STEM CELLS

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Purpose: Mesenchymal stem cells (MSCs) are an attractive cell source for cell therapy in osteoarthritis (OA) disease due to their capacity to differentiate towards chondrocytes and their skill to mobility and migration to the site of injury. To obtain more information about this disease, we have analyzed the protein expression profile of bone marrow MSCs of OA patients under chondrogenic differentiation using the SILAC (*Isotope Labeling by Amino Acids in Cell Culture*) technique.
Methods: Bone marrow cells were isolated from 3 OA patients and cultured in an expansion medium deficient in arginine and lysine. Regular L-lys (Lys0) and L-arg (Arg0) were added to the cell population harvested on day 2, whereas isotope-labeled L-lys (Lys6) and L-arg (Arg10) forms were put in the cell population harvested on day 14 of differentiation. After complete incorporation of the heavy amino acids, chondrogenic differentiation was induced by micromass culture under a commercial medium for 14 days. Expression of cartilage specific genes and histological assays were used to explore the chondrogenicity of MSCs. Proteins extracted from the two different time points were mixed 1:1 according to the measured protein concentrations, separated in 10% polyacrylamide gels and visualized using colloidal blue. Whole gel lines were cut into slices and subjected to in-gel digestion with trypsin. Separation of the resulting tryptic peptides was performed by nanoscale liquid chromatography coupled to mass spectrometry (nanoLC-MS/MS). The identification and quantification of proteins were carried out with Protein Pilot 2.0 software.
Results: Differentiation of MSCs was confirmed by inmunohistochemical detection of collagen II at day 14. Also, cells on day 2 and 14 show a progressive increase in orange staining for proteoglycans. Using metabolic labeling, we compared the proteomes at two different time points of their chondrogenic differentiation. We have identified and quantitatively analyzed 378 intracellular proteins. At least 46 of them appeared significantly regulated, including several glycolytic enzymes and structural proteins. This indicates that significant morphological and biological changes occurred during the 14 days of differentiation. 33 proteins were increased in the differentiated state compared to undifferentiated cells. Among them, we found a cartilage-specific protein, perlecan, which is important in the developmental processes that occur during chondrogenesis. In fact, it is able to influence extracellular matrix by stimulating collagen fibril formation.13 proteins exhibited a significantly reduced abundance from day 14 to day 2. Among them, we found Tropomyosin 4, a predominant regulator in actin filament